ISOZYME VARIATION IN SPECIES OF PROSOPIS (LEGUMINOSAE)[1]

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THE GENUS *Prosopis* — mesquite, algarrobo, screwbean — is comprised of about 40 species (Burkart, in press). They are found primarily in North and South America, but three species grow in East Africa and Asia Minor. The genus *Prosopis* consists mostly of small to large trees, although a few species of shrubs and woody perennial herbs are also known. They inhabit primarily subtropical areas with rainfall regimes of 200–800 mm. and a pronounced dry period. In areas with less than 250 mm. the species are restricted to washes or places with a high water table. Some species (e.g., *P. ruscifolia, P. glandulosa*) become obnoxious weedy trees, particularly where man is trying to preserve a grass cover in spite of heavy grazing by his own domestic animals (Morello *et al.*, 1971; Fisher, 1973). Other species (e.g., *P. tamarugo*) are being grown in different parts of the world (India, Chile) as forest species adapted to semiarid conditions.

Several species of *Prosopis* (*P. alba, P. nigra, P. chilensis, P. velutina,* etc.) are the principal riparian trees in the semidesert regions of the "Monte" (Morello, 1958) in Argentina, and the Sonoran Desert (Shreve, 1951) in the United States and Mexico. In conjunction with a comparative ecological study of these areas (Solbrig, 1972a), the genus *Prosopis* was singled out for an in-depth ecological and evolutionary study (Carman, 1973; Solbrig & Cantino, 1975). One of the objectives of the study was to investigate the genetic structure of populations of species of *Prosopis*. Specifically, we asked the following questions: (1) what is the level of genetic variation in species of *Prosopis* and (2) what is the extent of genetic differentiation among species and among populations of the same species? In this paper we attempt to answer these questions by presenting data on variation in isozymes of several species of *Prosopis*.

MATERIALS AND METHODS

Sampling. Twenty to 50 fruits of a tree selected at random were gathered at maturity before they dropped to the ground. Up to ten trees per population were sampled, although only one to three trees per population were used in the assays. The fruits of each tree were kept separate for most species, but they were mixed in a few cases. In the laboratory the

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[1] Contribution of the Structure of Ecosystem Program of the U.S./I.B.P.

seeds from the fruits were extracted by hand and 80 undamaged and well-developed seeds were selected for germination. The species sampled and the number of seedlings assayed are listed in TABLE 1. The samples comprise 13 of the 20–25 species in section ALGAROBIA as well as the two species of section CAVENICARPA. In most cases more than one locality was sampled. Altogether, 2145 seedlings belonging to 15 species from 48 localities were analyzed.

Experimental techniques. Seeds were germinated on plastic trays in loamy soil at field capacity at 20° C. The cotyledons of 8–10-day-old seedlings were used for electrophoretic analysis on starch gels. The methods used are those described in Brewer (1970) and modified by Solbrig (1972b). For each seedling, the following six enzymatic systems were assayed: α-esterase, LAP (lucine-amino-peptidase), ADH (alcohol-dehydrogenase), MDH (malic-acid-dehydrogenase), G6DH (glucose-6-phosphate-dehydrogenase), and catalase. The data from the last two systems were ambiguous and are not presented here.

Analysis of data. The isozyme data were analyzed in two ways. In the first method, we obtained the frequency of each isozyme band in the population being analyzed (all seedlings from a tree, or all seedlings from a locality, etc.) and calculated the diversity of the population using the Shanon-Weiner information measure:

$$H' = -\Sigma P_i \log_2 P_i$$

This formula measures the "evenness" of the distribution of the bands in the population. So, for example, if two alleles produce alternate bands, the highest value of H′ in the population is obtained when the bands have a 50:50 distribution, and the lowest value is obtained when the frequency of one of the bands approaches zero. In addition, the larger the number of bands, the higher the value of H′ is. In our context, a high value of H′ therefore signifies populations with many bands, each with a relatively high frequency.

Since different samples possess different numbers of bands (and presumably alleles), the ratio of H′ to H'_{max} (H'/H'_{max}) was calculated (where $H'_{max} = \log_2 P_i$). This expresses how close the sample is to a perfectly even distribution. Unfortunately, populations formed entirely by identical homozygous individuals will also have a H'/H'_{max} ratio of 1. Consequently, a second method of analysis was also used. In this case we scored the frequencies of all the possible combinations of bands (which we will call the "zymogram phenotypes") to get a measure of the number of genotypes in the population. Only the zymogram phenotype data are presented (TABLE 3), since they are representative of the results obtained with the other enzymes.

The number of segregating loci and the allelic frequencies in populations were not calculated, because the sample size was not large enough to produce accurate estimates and such a calculation would be misleading.

TABLE 1. Species and populations of Prosopis sampled.

SPECIES	COLLECTOR & SAMPLE NO.		LOCALITY	NO. OF SEEDLINGS ANALYZED
P. alba	*Solbrig*	*4238*	Argentina, Prov. Formosa, Bartolomé de las Casas	41
	Solbrig	*4272*	Argentina, Prov. Santiago del Estero, Quimilo	15
P. algarobilla	*Naranjo*	*355*	Argentina, Prov. Entre Rios	19
	Naranjo	*369*	Argentina, Prov. Entre Rios	44
	Naranjo	*365*	Argentina, Prov. Entre Rios	31
	Naranjo	*364*	Argentina, Prov. Entre Rios	5
P. alpataco	*Simon*	*449*	Chile, Prov. Santiago, Cuesta de Chacabuco	58
	Simon	*440*	Chile, Prov. Aconcagua, Los Andes	25
	Simon	*445*	Chile, Prov. Aconcagua, La Rinconada	25
	Simon	*461*	Chile, Prov. Santiago, Polpaico	7
P. caldenia	*Simpson*	*1001*	Argentina, Prov. La Pampa, Santa Rosa	39
	Hunziker	*9055*	Argentina, Prov. La Pampa	63
	Hunziker	*9052*	Argentina, Prov. La Pampa	62
P. chilensis	*Simon*	*456*	Chile, Prov. Santiago, Los Andes	26
	Simon	*429*	Chile, Prov. Santiago, Cuesta de Chacabuco	20
P. ferox	*Hunziker*	*s.n.*	Argentina, Prov. Salta, road to Antofagasta	20
P. flexuosa	*Simpson*	*1022*	Argentina, Prov. La Rioja, Arauco	43
	Hunziker	*9199*	Argentina, Prov. Catamarca, Andalgalá	44
	Hunziker	*9107*	Argentina, Prov. Catamarca, Andalgalá	50
	Simpson	*1025*	Argentina, Prov. Catamarca, Andalgalá	48
P. glandulosa	*Simpson*	*2218*	Texas, Hudspeth Co., McNary	142
	Simpson	*2217*	Texas, El Paso Co., Fabens	106
	Simpson	*2215*	New Mexico, Luna Co., Las Cruces	45
P. juliflora	*Hunziker*	*4716*	Colombia, Dept. Tolima, Honda	36
	Hunziker	*4715*	Venezuela, Mérida	24
	Solbrig	*4708*	Venezuela, Mérida, Lagunillas	20
P. laevigata	*Peñalosa*	*3427*	Mexico, San Luis Potosí, Ciudad del Maiz	32
	Peñalosa	*3433*	Mexico, San Luis Potosí, Tula	30
	Peñalosa	*3423*	Mexico, San Luis Potosí, Ciudad del Maiz	29
	Peñalosa	*3422*	Mexico, San Luis Potosí, Ciudad del Maiz	54
P. nigra	*Solbrig*	*4292*	Argentina, Prov. Córdoba, Monteros	26
	Solbrig	*4267*	Argentina, Prov. Formosa, Bartolomé de las Casas	90
	Solbrig	*4248*	Argentina, Prov. Formosa, Bartolomé de las Casas	66
	Solbrig	*4263*	Argentina, Prov. Formosa, Bazan	45
	Simpson	*1024*	Argentina, Prov. Catamarca, Andalgalá	41
P. ruscifolia	*Solbrig*	*4252*	Argentina, Prov. Formosa, Estanislao del Campo	68
	Solbrig	*4240*	Argentina, Prov. Formosa, Bartolomé de las Casas	37
	Solbrig	*4236*	Argentina, Prov. Formosa, Bartolomé de las Casas	46
	Solbrig	*4235*	Argentina, Prov. Formosa, Bartolomé de las Casas	32
	Solbrig	*4249*	Argentina, Prov. Formosa, Bartolomé de las Casas	44
P. sericantha	*Simpson*	*1015*	Argentina, Prov. La Rioja, Chamical	56
P. tamarugo	*Simon*	*171*	Chile, Prov. Tarapacá, Tarapacá	15
	Simon	*183*	Chile, Prov. Tarapacá, Tarapacá	15
	Simon	*173*	Chile, Prov. Tarapacá, Tarapacá	29
P. velutina	*Simpson*	*2200*	Arizona, Pima Co., Tucson	35
	Simpson	*2211*	Mexico, Sonora, Km. 557, Santa Ana	122
	Simpson	*2227*	Arizona, Pima Co., Ajo Way near Tucson	107
	Simpson	*2304*	Arizona, Maricopa Co., Gila Bend	84

TABLE 2. H' and H'/H'_{max} values for all populations of Prosopis studied.

SPECIES	COLLECTOR & SAMPLE NO.		α-ESTERASE		LAP		ADH		MDH	
			H'	H'/H'_{max}	H'	H'/H'_{max}	H'	H'/H'_{max}	H'	H'/H'_{max}
P. alba	*Solbrig*	*4238*	2.003	0.870	1.715	0.745	1.573	0.878	1.386	1.000
	Solbrig	*4272*	1.753	0.761	1.098	0.476	1.384	0.772	1.386	1.000
P. algarobilla	*Naranjo*	*355*	0.892	0.910	—	—	1.482	0.827	1.386	1.000
	Naranjo	*369*	1.870	0.899	—	—	1.562	0.872	1.386	1.000
	Naranjo	*365*	1.899	0.913	—	—	1.386	0.773	1.386	1.000
	Naranjo	*364*	1.777	0.854	—	—	1.386	0.773	1.386	1.000
P. alpataco	*Simon*	*449*	1.904	0.866	1.486	0.676	1.386	1.000	1.197	0.863
	Simon	*440*	2.027	0.922	1.776	0.808	1.386	1.000	1.377	0.993
	Simon	*445*	2.133	0.970	1.573	0.719	1.386	1.000	1.299	0.937
	Simon	*461*	1.863	0.847	1.361	0.619	1.386	1.000	1.231	0.888
P. caldenia	*Simpson*	*1001*	1.954	0.889	1.350	0.753	1.859	0.955	1.386	1.000
	Hunziker	*9055*	1.998	0.903	1.337	0.746	1.890	0.971	1.386	1.000
	Hunziker	*9052*	1.986	0.909	1.351	0.754	1.890	0.971	1.386	1.000
P. chilensis	*Simon*	*456*	1.881	0.817	1.374	0.767	1.386	0.773	1.098	0.792
	Simon	*429*	2.199	0.955	1.289	0.719	1.530	0.854	1.098	0.792
P. flexuosa	*Simpson*	*1022*	2.019	0.877	1.475	0.823	1.386	0.773	1.076	0.776
	Hunziker	*9199*	2.089	0.871	1.264	0.705	1.863	0.957	1.098	0.792
	Hunziker	*9107*	2.353	0.981	1.599	0.892	1.887	0.970	1.095	0.790
	Simpson	*1025*	1.686	0.703	1.597	0.891	—	—	1.271	0.917
P. glandulosa	*Simpson*	*2218*	2.000	0.869	—	—	1.406	0.873	1.386	1.000
	Simpson	*2217*	1.949	0.847	—	—	1.410	0.876	1.386	1.000
	Simpson	*2215*	1.997	0.847	—	—	1.386	0.861	1.386	1.000
P. juliflora	*Hunziker*	*4716*	1.834	0.796	1.679	0.937	1.386	1.000	1.386	1.000
	Hunziker	*4715*	1.791	0.778	1.098	0.613	1.386	1.000	1.386	1.000
P. laevigata	*Peñalosa*	*3423*	2.035	0.884	1.098	1.000	1.386	1.000	1.386	1.000
	Peñalosa	*3422*	2.049	0.890	1.098	1.000	1.386	1.000	1.386	1.000
	Peñalosa	*3427*	—	—	1.098	1.000	—	—	1.386	1.000
	Peñalosa	*3433*	—	—	1.098	1.000	—	—	1.386	1.000
P. nigra	*Simpson*	*1024*	—	—	1.530	0.664	—	—	1.098	0.792
	Solbrig	*4292*	—	—	1.335	0.579	—	—	—	—
	Solbrig	*4263*	2.096	0.954	1.918	0.833	1.644	0.845	1.307	0.943
	Solbrig	*4267*	1.951	0.847	1.661	0.721	1.670	0.858	1.133	0.817
	Solbrig	*4248*	—	—	1.743	0.679	—	—	—	—
P. ruscifolia	*Solbrig*	*4252*	1.634	0.785	1.098	0.428	1.527	0.785	1.098	1.000
	Solbrig	*4240*	1.484	0.712	1.728	0.673	1.479	0.760	1.098	1.000
	Solbrig	*4236*	1.564	0.752	1.859	0.739	1.386	0.712	1.098	1.000
	Solbrig	*4235*	1.690	0.812	1.369	0.533	1.386	0.712	—	—
	Solbrig	*4249*	—	—	1.554	0.606	—	—	1.098	1.000
P. sericantha	*Simpson*	*1015*	2.044	0.930	0.864	0.786	1.857	0.845	1.098	1.000
P. tamarugo	*Simon*	*171*	1.096	1.000	1.098	1.000	1.386	1.000	0.693	1.000
	Simon	*183*	1.096	1.000	1.098	1.000	1.386	1.000	0.693	1.000
	Simon	*173*	1.096	1.000	1.098	1.000	1.386	1.000	0.693	1.000
P. velutina	*Simpson*	*2200*	2.047	0.934	1.381	0.858	1.786	0.918	0.693	1.000
	Simpson	*2211*	2.053	0.937	1.385	0.860	1.782	0.916	0.693	1.000
	Simpson	*2227*	2.038	0.931	1.374	0.853	1.788	0.919	0.693	1.000
	Simpson	*2204*	2.121	0.965	1.355	0.842	1.883	0.911	0.693	1.000

RESULTS

DIVERSITY AND VARIATION WITHIN SAMPLES

α-esterase. The number of isozyme bands varied from three to 11 in different species. In most species, two zones of activity can be distinguished: zone 1, with two to four bands, and zone 2, with four to six bands. Bands of zone 1 are thicker and more intensely stained than bands of zone 2. The exception is band 5 of zone 2, which in some species is as intensely stained as the bands of zone 1. The expression of zone 2 bands appears to be modified by minor variations in experimental techniques; occasionally, seedlings from the same sample showed different numbers of zone 2 bands with no consistent pattern from one day to the next. Although such samples were excluded from the data presented here, we are uncertain about the reliability of data on the frequency of zone 2 bands as a whole. Consequently, we have not used such data to make intra- or interspecific comparisons.

Samples of all species showed high H'/H'_{max} ratios (TABLE 2). However, samples of some species had higher ratios than others. For example, in *Prosopis ruscifolia* the H'/H'_{max} ratio ranged from 0.712 to 0.812, whereas in *P. velutina* the range was from 0.931 to 0.965 (TABLE 2).

Samples of all species except *Prosopis juliflora* and *P. tamarugo* showed a large number of zymogram phenotypes (TABLE 3). The frequency of the common classes differed from one species to another. For example, in *P. juliflora* 85% of the individuals sampled belong to one phenotype (TABLE 3). In the other extreme, not more than 35% of the individuals of *P. velutina* belong to one given class.

LAP. The LAP isozymes also appear in two different zones. Zone 1 has one to four bands, which are intensely stained. Most species have one or two bands in this region. The zone 2 bands, which are thin and faintly stained, can be further subdivided into two groups: the slow migrating subgroup *a* and the fast migrating subgroup *b*, with one or two bands in each.

In all species, the samples had a high H'/H'_{max} ratio. There was much variation within samples with respect to the number and frequency of various zymogram phenotypes.

ADH. The ADH isozymes separate into three zones. Most species show only two zones of activity. The number of bands in zone 1 and zone 2 vary from two to three, in zone 3 from two to five.

The diversity measures for ADH bands were generally higher than those for α-esterase and LAP bands (TABLE 2). Again, samples of some species had higher H'/H'_{max} values than other species. For example, the H'/H'_{max} ratio for *Prosopis algarobilla* ranges from 0.773 to 0.872, while for *P. alpataco*, *P. laevigata*, and *P. tamarugo* the ratio is 1.00, indicating that these last species are probably homozygous for various ADH loci.

In contrast to the situation in α-esterase and LAP, the number of band phenotypes obtained for ADH isozymes within each sample was remarkably uniform throughout. The total number of bands considered for phenotypes was greater than the total for α-esterase isozymes, and almost equal to the total for LAP. Samples of species such as *Prosopis alpataco*, *P. juliflora*, *P. laevigata*, and *P. tamarugo* had only one class of phenotypes for all samples. In *P. algarobilla*, *P. chilensis*, *P. glandulosa*, *P. nigra*, and *P. ruscifolia*, 70 to 90% of the individuals within samples belonged to one class. Only in *P. alba*, *P. flexuosa*, and *P. velutina* did the individuals within samples occur in two or more classes in almost equal proportions.

MDH. All MDH isozymes separate in the same zone and their numbers vary from two to four in different species. Except for *Prosopis alpataco*, *P. chilensis*, *P. flexuosa*, and *P. nigra*, the $H'/H'_{\max}$ ratio in samples of all species was 1.00 (TABLE 2).

The level of variation displayed by the distribution of different zymogram phenotypes within the samples was extremely low. The number of phenotypes was rarely more than two in most species and, furthermore, a majority of phenotypes belonged to one class.

DIVERSITY AND VARIATION BETWEEN SAMPLES OF THE SAME SPECIES

α-esterase. None of the species investigated showed significant differences between samples with respect to $H'/H'_{\max}$ ratios. However, in regard to the zymogram phenotypes of the first four bands, some differences were observed among samples in a few species. For example, one sample of *Prosopis alpataco* (*445*) showed about one-fourth of its individuals with bands 1 and 2, which were completely lacking in the three other samples of the species. In *P. caldenia*, sample *9055* showed a high frequency of individuals with bands 1, 2, 3, and 4. However, population samples *9052* and *1001* had such individuals in low frequency. Instead, they had a large number of individuals with bands 3 and 4 (TABLE 3). Other species showing such differences between population samples were *P. algarobilla* (with bands 2, 3, and 4) and *P. chilensis* (with bands 1, 2, 3, and 4) (TABLE 3).

LAP. In contrast to the situation in α-esterase, there was considerable variation among samples of several species with respect to $H'/H'_{\max}$ ratios. Such species include *Prosopis alba*, *P. alpataco*, *P. juliflora*, *P. nigra*, and *P. ruscifolia* (TABLE 2). Similarly, many species showed much variation among samples with respect to zymogram phenotypes. Because of a possibility that some of the observed variation is the result of experimental techniques, none of these cases are described in detail.

ADH. The $H'/H'_{\max}$ ratios were remarkably uniform among samples of all species. However, with respect to phenotypes based on zymograms, samples in several species differed in the frequency of various phenotypes. For example, in *Prosopis chilensis* only population sample *429* had more

TABLE 3. Frequency of various α-esterase patterns in Prosopis species (in %).

SPECIES	COLLECTOR & SAMPLE NO.		N	PATTERNS OF BANDS 4	1,2	3,4	2,3,4	1,2,3,4	OTHER
P. alba	*Solbrig*	*4238*	41	0.243	—	0.487	—	0.048	0.222
	Solbrig	*4272*	15	—	—	1.000	—	—	—
		TOTAL	56	0.178	—	0.660	—	0.035	0.127
P. algarobilla	*Naranjo*	*369*	44	—	—	0.022	0.159	—	0.819
	Naranjo	*364*	5	—	—	—	0.400	—	0.600
	Naranjo	*355*	19	—	—	—	0.894	—	0.105
	Naranjo	*365*	31	—	—	—	0.258	—	0.741
		TOTAL	99	—	—	0.009	0.343	—	0.646
P. alpataco	*Simon*	*449*	58	—	—	0.775	0.019	0.206	—
	Simon	*440*	25	—	—	0.200	0.520	0.040	0.240
	Simon	*445*	25	—	0.240	0.480	—	0.200	0.080
	Simon	*461*	7	—	—	0.428	—	—	0.572
		TOTAL	115	—	0.052	0.565	0.121	0.156	0.106
P. caldenia	*Hunziker*	*9052*	62	—	—	0.580	0.370	—	—
	Hunziker	*9055*	63	—	—	0.822	0.177	—	—
	Simpson	*1001*	39	—	—	0.743	0.179	—	—
		TOTAL	164	—	—	0.711	0.250	—	—
P. chilensis	*Simon*	*456*	26	0.076	—	0.730	0.153	—	0.038
	Simon	*429*	20	—	0.050	0.400	—	0.250	0.300
		TOTAL	46	0.043	0.022	0.587	0.081	0.109	0.152
P. glandulosa	*Simpson*	*2218*	142	0.128	—	0.727	—	0.136	0.083
	Simpson	*2217*	106	0.009	—	0.764	0.122	0.009	0.084
	Simpson	*2215*	45	0.044	—	0.777	0.044	0.066	0.069
		TOTAL	293	0.070	—	0.749	0.053	0.077	0.081
P. juliflora	*Hunziker*	*4716*	36	—	—	—	1.000	—	—
	Hunziker	*4715*	24	—	—	—	1.000	—	—
		TOTAL	60	—	—	—	1.000	—	—
P. laevigata	*Peñalosa*	*3423*	29	—	0.344	0.206	—	0.379	0.068
	Peñalosa	*3422*	54	—	0.166	—	—	0.500	0.333
		TOTAL	83	—	0.313	0.171	—	0.400	0.113
P. nigra	*Solbrig*	*4263*	45	—	—	0.129	0.451	—	0.420
	Solbrig	*4267*	90	0.111	—	—	0.788	0.011	—
		TOTAL	135	0.086	—	0.034	0.734	0.008	0.140
P. ruscifolia	*Solbrig*	*4252*	68	0.985	—	—	0.014	—	—
	Solbrig	*4240*	37	1.000	—	—	—	—	—
	Solbrig	*4236*	46	1.000	—	—	—	—	—
	Solbrig	*4235*	32	1.000	—	—	—	—	—
	Solbrig	*4249*	44	0.272	—	0.136	0.545	—	0.057
		TOTAL	227	0.854	—	0.026	0.110	—	0.011
P. sericantha	*Simpson*	*1015*	56	—	—	0.035	0.767	0.178	0.020
P. velutina	*Simpson*	*2200*	35	—	0.354	0.193	0.193	0.161	0.096
	Simpson	*2211*	122	—	0.254	0.327	0.016	0.270	0.133
	Simpson	*2227*	107	0.037	0.355	0.196	—	0.280	0.291
		TOTAL	264	0.015	0.308	0.256	0.033	0.259	0.192

than 25% of its individuals with bands 6, 7, 9, and 11. In *P. velutina*, population sample *2227* had about 25% of its individuals with bands 7, 8, 11, and 12, and about 65% with bands 6, 7, 9, 10, and 11. On the other hand, population samples *2211* and *2204* had more than 50% of their individuals with bands 7, 8, 11, and 12 and about 30% with bands 6, 7, 10, and 11.

MDH. There were no appreciable differences between population samples either with respect to H'/H'_{max} ratios or with respect to zymogram phenotypes.

DIVERSITY AND VARIATION BETWEEN DIFFERENT SPECIES

The various species differed from each other in two respects: (a) frequency and electrophoretic mobility of isozyme bands and (b) levels of variation.

α-esterase. *Prosopis tamarugo* is different from all other species because it has only three isozyme bands. Although these are numbered 4, 5, and 6 in TABLE 2, they migrated at a slightly faster rate than the equivalent bands of other species. *Prosopis ruscifolia* differs from other species in that bands 1, 2, and 3 either occur in very low frequency (less than 10%) or are completely absent. The zymograms of *P. juliflora* lack bands 1, 5, and 7, and instead appear to be fixed for bands 2, 3, 4, 6, 8, and 9. Among the closely related South American species *P. alpataco*, *P. chilensis*, *P. caldenia*, *P. algarobilla*, *P. alba*, and *P. nigra* no appreciable differences were observed. However, among the closely related North American species *P. glandulosa* and *P. velutina* there are notable differences in the frequency of certain bands. In *P. glandulosa* bands 1 and 2 occur in low frequency (less than 25%), while in *P. velutina* they occur in high frequency (more than 50%).

In terms of levels of variation, two groups of species can be distinguished. One, comprised of *Prosopis tamarugo*, *P. juliflora*, and *P. ruscifolia*, shows very little variation. The other, involving the rest of the species, is highly variable.

LAP. Except for *Prosopis alba*, *P. nigra*, *P. glandulosa*, and *P. velutina*, which had similar zymograms, all species showed differences in the location and frequency of one or more bands. Most species were quite variable in terms of H′, with the sole exception of *P. tamarugo* (TABLE 2).

ADH. *Prosopis sericantha* has zymograms that are quite distinct from the remainder of the species. *Prosopis tamarugo*, which differs from all the other species investigated in the patterns of α-esterase, LAP, and MDH bands, does not differ with respect to the patterns of ADH bands. *Prosopis ruscifolia*, a species quite distinct from the others analyzed in respect to the patterns of α-esterase and LAP bands, also shows a characteristic species-specific ADH zymogram, with band 8 in almost 100% frequency in all samples studied. In the other South American species this band, if it is present at all, occurs in very low frequency (3% or less). Exceptions are *P. caldenia* and *P. flexuosa*, where the frequency of band 8 may vary from 25% in the former to 50% in the latter. Among other closely related South American species, *P. caldenia* and *P. flexuosa* differ from the remainder in having not only band 8, but also bands 11 and 12 in relatively high frequencies (TABLE 2). Among the three closely related North American species analyzed, *P. velutina* differs from *P. glandulosa* and *P. laevigata* in having bands 6, 9, and 10 in very high frequencies (more than 50%). These bands are totally absent in the other two species.

On the basis of levels of variation, the species can be included in one of two groups. The first includes *Prosopis tamarugo*, *P. ruscifolia*, *P. chilensis*, *P. juliflora*, *P. algarobilla*, *P. nigra*, *P. alba*, *P. laevigata*, and *P. glandulosa*. All or more than 95% of the individuals have the same phenotype. The second group, containing *P. sericantha*, *P. caldenia*, *P. flexuosa*, and *P. velutina*, is more variable than the first; most of the individuals in these species are more or less evenly distributed among two or three phenotypes.

MDH. *Prosopis tamarugo* again has very distinct zymograms. These differ from other species in having bands 5 and 6, which are totally absent in all other species. *Prosopis sericantha*, with bands 1, 2, and 3, also has zymograms which appear to be species-specific. *Prosopis ruscifolia* and *P. chilensis* differ from the other South American species in lacking band 1. Along the same lines, *P. alpataco* and *P. flexuosa* can be distinguished from the rest, since they have band 1 in very low frequency. The North American species, *P. glandulosa*, *P. velutina*, and *P. laevigata*, are all alike, but they differ from the South American species in having bands 1 and 2 very faintly stained.

All species are remarkably uniform in their MDH isozyme patterns and show no differences with respect to levels of variation.

DISCUSSION

Although only four enzyme systems were analyzed, we believe the number of loci controlling the expression of various isozyme bands for the four systems to be more than ten. Thus the number of loci examined in the present study is fairly large for studies of this kind, and on the basis of data already presented, it is reasonable to suggest that the species of *Prosopis* are genetically quite diverse. The progeny of all species of *Prosopis* investigated segregated for one or more enzymatic loci, indicating the existence of heterozygous individuals. The level of genetic diversity observed in various species is higher than that of some species of inbreeding herbs, such as *Avena barbata* (Jain, 1969) and species of *Leavenworthia* (Solbrig, 1972b). This is to be expected, for although precise information about breeding systems of *Prosopis* species is lacking, the taxa analyzed are considered to be widely outcrossed (Simpson, in preparation).

One could argue that the observed levels of variation are overestimates of the actual levels of variation in populations, since the analyzed seedlings were not subjected to selection. This might be true. However, it should be pointed out that the estimates presented here take into account not only the diversity of zymogram phenotypes, but also the number of segregating bands. The allelic diversity at a locus among the seedlings cannot arise unless such a diversity is also present in the population of adults.

In regard to differences among species with respect to isozyme variability, two points deserve attention. It should first be noted that species which belong to a different section of the genus, e.g., *Prosopis sericantha* and *P. alpataco*, have vastly different zymograms for various enzyme systems; furthermore, within a section (as already discussed), some closely related species show minor differences in zymograms. The second and perhaps more important point is that some of the species and populations showed less variation than others. Three species in particular can be singled out: *P. juliflora*, *P. tamarugo*, and *P. ruscifolia*.

Three populations of *Prosopis juliflora* were investigated, one from western Colombia and two from western Venezuela. They are remarkably similar, particularly if one considers that they are separated from each other by several hundred kilometers, and more importantly, that they are separated by areas of high mountains and tropical vegetation, where *Prosopis* does not grow. Most of the plants investigated showed a remarkably uniform pattern of several (more than three) nonsegregating bands. So, for example, all 56 plants showed the same six bands for α-esterase (bands 2, 3, 4, 6, 8, and 9), although bands 10 and 11 also appeared at low frequencies in population *4716* from Colombia. Population *4715* from Venezuela showed no variability in the LAP pattern and all plants had bands 2, 3, and 5. All plants of the Colombian population were fixed for bands 5, 6, and 8, but segregated for bands 2 and 3. For MDH both populations showed the same invariable pattern of four bands, and a similar situation was also found for ADH.

The genetics of the ADH locus were investigated in more detail, and we were able to show that *P. juliflora* is a permanent heterozygote for ADH, with the genetic constitution S^2S^2, F^2F^2/S^3S^3, F^3F^3, possibly due to gene duplication. Although the patterns for α-esterase, LAP, and MDH cannot be analyzed with the same precision as the ADH locus, the similarity in the zymograms suggests that there may be some fixed heterozygosity in these systems as well (Bawa & Solbrig, in preparation).

Prosopis juliflora grows in isolated, arid, rain-shadow valleys in Colombia and Venezuela (and in the Caribbean and Mexico). These valleys are surrounded by very high mountains (3,000 m. and higher) and are separated from each other by several hundred kilometers. There is evidence that some, if not most, of these dry pockets are post-Pleistocene in origin, and most of these populations are around 10,000 years old or less. Given the large size of *Prosopis* seeds, gene flow between populations is a very rare event indeed. Seeds are carried in the digestive tracts of animals (Solbrig & Cantino, 1975), and there is anecdotal evidence that the species has spread in Colombia with the development of the cattle industry. This special ecological circumstance (with populations being started by one or a few seeds) subjects the species to repeated events of severe genetic depletion. Under these conditions, mechanisms such as gene duplication that can create permanent heterozygotes may be favored.

Prosopis tamarugo is a species of the very dry coastal areas of the province of Tarapacá in northern Chile. Of all the species investigated, it grows in the most extreme environment. Three population samples were investigated. They were characterized by the lowest total number of isozyme bands of any species investigated and the lowest within-sample variation. A single sample of a related species, *P. ferox* (from an equally extreme environment in the high Puna of Argentina), was analyzed and was also found to be invariable and to have very few bands. It is our contention that in extreme environments selection may favor certain particular superior genotypes, and this may help to explain the lowered variability of these species.

Finally, we would like to mention *Prosopis ruscifolia*, the "vinal" from Argentina, and *P. glandulosa*, the "honey mesquite" from Texas. Both of these species (Morello *et al.*, 1971; Fisher, 1973) have become obnoxious agricultural pests in grassland areas. In both, but particularly in *P. ruscifolia*, we detected a slightly lower degree of genetic variability than the mean for all species. This could be attributed to the fact that these are expanding populations (Morello *et al.*, 1971; Fisher, 1973).

The significance of observed differences in variation patterns among samples of *Prosopis alpataco*, *P. caldenia*, *P. algarobilla*, and *P. chilensis* for α-esterase isozymes and those of *P. chilensis* and *P. velutina* for ADH isozymes is obscured by the fact that only a few samples, consisting of a small number of trees, were analyzed. Interpopulation differences with respect to isozymes have been observed as well in some coniferous trees (Clarkson & Fairbrothers, 1970). However, because of differences in techniques of different investigators, it is not possible to say whether the differences reported here are comparable to those reported for conifers.

In summary, then, species of *Prosopis* are characterized by a high degree of genetic variability. Within the genus two species show significantly lower levels of variability. This can be attributed to severe genetic depletion because of extreme reduction of the population in one case, and to extreme directional selection in the other.

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THE BALSAMINACEAE IN THE SOUTHEASTERN UNITED STATES[1]

Carroll E. Wood, Jr.

BALSAMINACEAE A. Richard, Dict. Class. Hist. Nat. 2: 173. 1822, "Balsamineae," nom. cons.

(Touch-me-not Family)

Annual [or perennial] herbs [sometimes epiphytic, or subshrubs] with simple, alternate [opposite or whorled] exstipulate leaves. Flowers perfect, protandrous, zygomorphic, axillary, solitary, or clustered. Sepals 3 [5], the apparent lowest or posterior one (the flowers resupinate) ± saccate and [usually] spurred, the spur nectariferous, the 2 lateral sepals free [or connate]. Petals 5 [free] or by connation of the lateral petals in pairs appearing to be 3. Stamens 5, alternate with the petals; filaments short, free or ± connate, in ours with a scalelike appendage on the inner side, the scales partly united over the gynoecium; anthers 2-locular [coherent or] connivent, opening by a slit or pore. Gynoecium [4- or] 5-carpellate, syncarpous; stigmas 5, minute, sessile [or style short, subulate, stigma 1]; ovary superior [4- or] 5-loculate, each locule with 2 to many anatropous, 2-integumented ovules, the placentation axile. Fruit a ± fleshy [4- or] 5-valved capsule, usually opening elastically, or (in *Tytonia*) drupaceous or a fleshy capsule(?). Seeds with a straight embryo and without endosperm. Type genus: *Balsamina* P. Miller, nom. illegit. = *Impatiens* L.

A family of three genera and some 500 described species. *Tytonia* G. Don (*Hydrocera* Blume ex Wight & Arnott) comprises only a single species, *T. triflora* (L.) C. E. Wood (*Hydrocera triflora* (L.) Wight &

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[1] Prepared for the Generic Flora of the Southeastern United States, a joint project of the Arnold Arboretum and the Gray Herbarium of Harvard University made possible through the support of the National Science Foundation, currently under Grant BMS74-21469 (Carroll E. Wood, Jr., principal investigator). This treatment, the seventy-sixth in the series, follows the pattern established in the first paper (Jour. Arnold Arb. **39**: 296-346. 1958) and continued to the present. The area covered by the Generic Flora includes North and South Carolina, Georgia, Florida, Tennessee, Alabama, Mississippi, Arkansas, and Louisiana. The descriptions apply primarily to the plants of this area, with supplementary information in brackets. References that the author has not verified are marked with an asterisk.

A preliminary paper on the Balsaminaceae written by Dr. K. A. Wilson for the Generic Flora project has been consulted in the preparation of this treatment. Beverly Vincent has aided in the preparation of the bibliography and the typing of the manuscript. The illustration was drawn by A. D. Clapman under the direction of the author. The material for the drawing of *Impatiens pallida* was collected by R. B. Channell and H. F. L. Rock.

Arnott),[2] $2n = 16$, of marshes from India to Java. *Semeiocardium* Zoll. is also monotypic, including only *S. Arriensii* Zoll., known solely from Madura and Kangean islands off the northeast coast of Java. *Impatiens* L. comprises the remainder of the species.

The family is a distinct one, characterized by the simple, exstipulate leaves, watery sap, resupinate zygomorphic flowers with a usually spurred often saccate sepal, stamens that are coherent around the gynoecium, and (in *Impatiens* and *Semeiocardium*) elastic dehiscence of the capsule. The fruit of *Tytonia* has been variously described as a berry; or as succulent, drupaceous, 5-angled, 5-furrowed, 5-loculate, the endocarp hard and bony, the seeds solitary (Wight & Arnott, p. 140); or as a 5-pyrenous drupe, "the stones 3–4 celled; only 1–2 cells seed-containing; the other ones empty and serving as a floating apparatus" (Backer & Bakhuizen, Fl. Java); or most recently as a purplish-red capsular berry dehiscing septicidally from the top leaving the seed attached to the column (Venkateswarlu & Dutt).

In *Tytonia* the perianth consists of five sepals (one spurred) and five petals, the lowermost largest. In *Impatiens* there are usually only three sepals (one saccate and usually spurred and two smaller lateral ones), but some species (cf. Hooker) have five, the two additional ones smaller than the others. The uppermost petal is free (and is often carinate or bifid), and the four others are connate in pairs, the pairs free from each other, each usually giving the appearance of a single two-lobed petal, but in *I. Wallerana* free nearly to the base and equal in size. An earlier interpretation of the perianth as composed of four sepals (the fourth [the uppermost petal in the above interpretation] being petaloid and often notched, presumably representing two sepals) and four petals (united in pairs) seems unlikely in view of the 10 perianth segments of *Tytonia* and the five sepals of some species of *Impatiens*. The perianth of *Semeiocardium* is composed of three sepals and five petals as in *Impatiens*, but the two fused petal-pairs have become connate into a bifid two-clawed liplike structure. *Semeiocardium* differs further from *Tytonia* and *Impatiens* in the largely connate lateral sepals, the 4-locular ovary, and the short, subulate style with a single stigma. Although it was originally described by Zollinger as a member of the Balsaminaceae, first Hasskarl and then Chodat mistakenly assigned it to the Polygalaceae. However, Backer's observations on living plants show that it is closely allied with *Impatiens* (cf. Miller, Jour. Arnold Arb. **52**: 273. 1971).

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[2] As Backer & Bakhuizen van den Brink (Fl. Java **1**: 251. 1963) pointed out a dozen years ago, Blume (Bijdr. Fl. Nederl. Indië 241. 1825) did not publish the generic name *Hydrocera* validly, since he gave only a family description (Hydrocereae) and neither a generic description nor a specific one that might serve as a combined generic-specific description. *Hydrocera* was first validly published by Wight & Arnott (Prodr. Fl. Penin. Indiae Orient. 140. 1834), but they cited *Tytonia* G. Don (Gen. Syst. Garden. Bot. **1**: 749. 1831) in synonymy, making *Hydrocera* superfluous and illegitimate. The single species in this genus should be known as **Tytonia triflora** (L.) C. E. Wood, comb. nov., basionym, *Impatiens triflora* L. Sp. Pl. **2**: 938. 1753.

The Balsaminaceae have been considered to be allied with the Geraniales, and they have even been included in the Geraniaceae, but neither position is supported by recent studies. Most recent students have placed the family with the Sapindales, but this association is also uncertain. Erdtman notes that "an affinity with Celastrales, Geraniaceae, Oxalidaceae, Simaroubaceae, or Violaceae has been suggested but is not supported by pollen morphology."

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